Specimens from a Vesicular Lesion Caused by Molluscum Contagiosum Virus Produced a Cytopathic Effect in Cell Culture That Mimicked That Produced by Herpes Simplex Virus

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Infection with molluscum contagiosum virus, a poxvirus, normally has a typical clinical presentation; therefore, laboratory confirmation is infrequently sought and the virus is rarely isolated in culture. As reported herein, viral culture of specimens from atypical lesions may produce an abortive infection in limited cell lines and a cytopathic effect suggestive of herpes simplex virus.

CASE REPORT

An 18-year-old woman presented to her health care provider with a 1-month history of a rash in the pubic area. The lesions were not painful and the patient had no history of similar dermatological problems. She stated that her husband had a similar lesion on his upper inner thigh. No unusual travel, exposure, or social history was reported. Upon examination, the patient had a single, nontender lesion in the mons area that had a vesicular herpetic appearance and multiple lesions in the groin area resembling folliculitis.

The provider suspected herpes simplex virus (HSV) infection and submitted a swab specimen collected from the lesion and placed in viral transport medium (Microtest M4-RT; Remel, Lenexa, KS) to the medical center virology laboratory for culture. A 750-μl aliquot of the specimen was removed from the viral transport medium and placed in a separate tube. An antimicrobic suspension of penicillin (1,000 U/ml), streptomycin (1,000 μg/ml), and amphotericin B (Fungizone [2.5 μg/ml]; Sigma-Aldrich, St. Louis, MO) was prepared. Two hundred microliters of the antimicrobic solution was added to the 750-μl aliquot of specimen, and the mixture was centrifuged for 10 min at 2,300 rpm (900 × g). Two-hundred-microliter aliquots of the processed specimen were inoculated into MRC-5 (Diagnostic Hybrids, Inc., Athens, OH) and A549 (Diagnostic Hybrids, Inc., Athens, OH) tube cultures and incubated at 36°C. Discrete foci of ballooning or rounding fibroblasts suggestive of HSV were observed at 24 h in the MRC-5 cells (Fig. 1); however, no cytopathic effect (CPE) was observed in the A549 cells. Attempts to pass the observed CPE to a subsequent tube of MRC-5 cells from the primary culture were unsuccessful, which might indicate specimen toxicity; however, no such effects were observed in A549 cells. The abortive CPE, which resembled the CPE of HSV, resulted in the death of the MRC-5 cells and was reproduced twice more using the original specimen in MRC-5 cells, while inoculated A549 cells were unaffected.

Cells from the primary MRC-5 cell culture exhibiting a CPE were trypsinized and fixed to slides for examination by fluorescent-antibody (FA) testing by two separate laboratories (Tripler Army Medical Center and Hawaii State Department of Health) using different products. The results of testing specific for HSV types 1 and 2 (the laboratories used reagents obtained from PathD$_x$ Diagnostic Products Corp., Los Angeles, CA, and Syva Co., Palo Alto, CA, respectively), varicella-zoster virus (VZV) (the laboratories used reagents obtained from Meridian Diagnostics, Inc., Cincinnati, OH, and Chemicon International, Inc., Temecula, CA, respectively), and cytomegalovirus (CMV) (the laboratories used reagents obtained from PathD$_x$ Diagnostic Products Corp., Los Angeles, CA, and Chemicon International, Inc., Temecula, CA, respectively) were all negative. FA testing for enteroviruses conducted at Tripler Army Medical Center (reagents obtained from Chemicon International, Inc., Temecula, CA) was also negative.

PCR testing of the primary culture material in our laboratory was negative for VZV and non-variola orthopoxvirus (reagents for the PCR were obtained from Laboratory Response Network for Bioterrorism Preparedness, Centers for Disease Control and Prevention, Atlanta, GA). A herpes simplex virus-specific PCR assay conducted at a reference laboratory (Mayo Medical Laboratories, Rochester, MN) was also negative for HSV types 1 and 2.

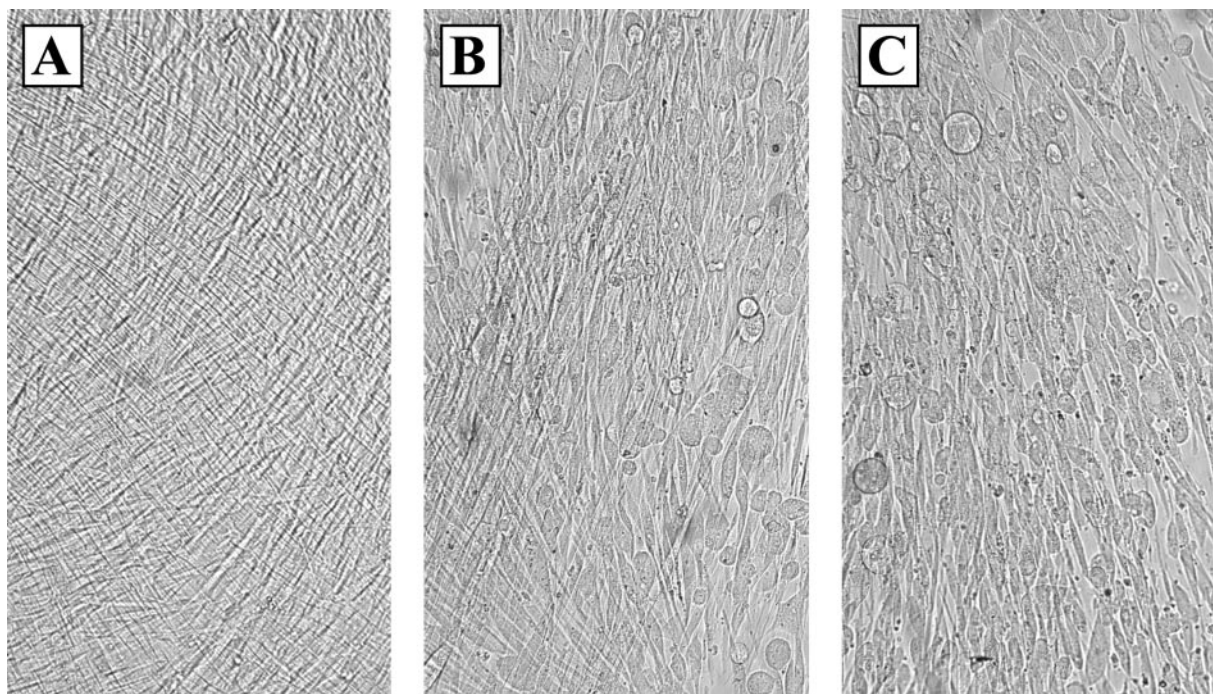Primary MRC-5 tube cultures were also processed for elec-

FIG. 1. MRC-5 cultures after 24 h of incubation. Comparison of uninfected (A) versus molluscum contagiosum virus-infected (B) and herpes simplex type 2 virus-infected (C) MRC-5 cells. Magnification, ×200.

tron microscopy after 24 h of incubation. Briefly, the tubes were vortexed and the cell suspension was centrifuged at 12,000 rpm (16,000 × g) for 5 min. The supernatant was removed and replaced with 500 μl of gluturaldehyde. The pellet was gently resuspended in the gluturaldehyde and centrifuged at 12,000 rpm (16,000 × g) for 1 min. The supernatant was again removed and replaced with 500 μl of fresh gluturalde-hyde, and the new pellet was stored, without resuspension, at 4°C prior to preparation and staining for electron microscopic examination according to standard methods (12). Transmission electron microscopy revealed the presence of large oval or brick-shaped virions that measured approximately 280 nm long by 220 nm wide (Fig. 2). The virion morphology and size were typical of poxviruses and consistent with those of molluscum contagiosum virus (MCV).

Molluscum contagiosum virus is the only poxvirus in the genus *Mollusciopoxvirus*. The other three genera of poxviruses known to infect humans include the *Orthopoxvirus*, *Parapoxvirus*, and *Yatapoxvirus* (9). MCV occurs world-wide, with a rel-atively high prevalence in tropical areas, and primarily affects children, sexually active adults, and immunocompromised per-sons (9). Transmission of MCV is primarily via direct skin-to-skin contact, indirect contact via fomites such as washcloths or towels, and autoinoculation. The incubation period varies widely, from 1 week to several months. Molluscum contagio-sum virus lesions in immunocompetent hosts eventually re-solve on their own but may persist for months if left untreated. Treatment is often recommended due to the potential for person-to-person transmission and autoinoculation (2). Treat-
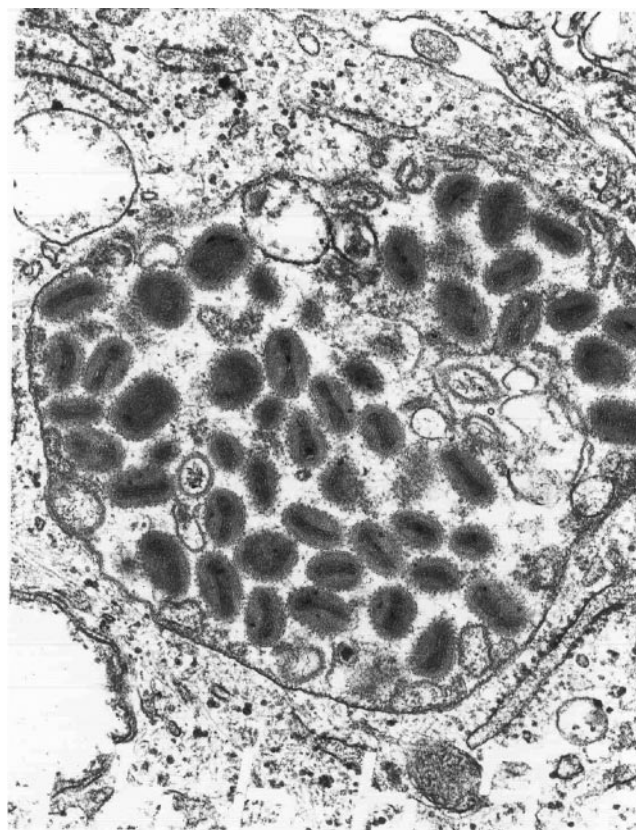

FIG. 2. Transmission electron micrograph of mature poxvirus par-ticles recovered from primary MRC-5 culture after 24 h of inoculation of patient specimen. Particles stained with uranyl acetate and lead citrate. Magnification, ×47,300.

ment consists of destructive methods such as curettage, cryotherapy, application of a chemical peeling agent, or the use of topical imiquimod cream (2, 13). Disseminated and intractable disease may occur in immunocompromised patients.

Clinical diagnosis of MCV infection is normally uncomplicated because the painless, waxy, and umbilicated or dimpled papules are quite typical (9, 11, 13). The differential diagnosis of the cause of these skin lesions includes other viral agents such as *Papillomavirus*, HSV, and VZV, various bacterial and fungal organisms, and noninfectious etiologies such as carcinoma or granuloma. MCV lesions normally group in one or two areas and can be anywhere on the body, although they rarely appear on palms, soles, or mucous membranes and are not associated with systemic symptoms (13). Lesions may have a diffuse distribution on the skin of children and immunocompromised persons (17). Depending on the anatomical location of the MCV papules, epidemiological risk factors, unrelated symptoms, and the availability of rapid electron microscopy services, it is possible that lesions could be confused with monkeypox or, during heightened concerns of bioterrorism, smallpox (1). Key differences in variola virus infection include lesions that are widespread, progress from macules to papules to vesicles to pustules to crusts, and are associated with severe clinical symptoms.

Laboratory confirmation of MCV infection is rarely requested but can be accomplished with histological examination of lesion biopsy specimens that reveals characteristic molluscum inclusion bodies (6, 17). The diagnosis may also be confirmed by electron microscopy (6) and PCR (16, 18). Laboratory testing is most often conducted on lesions from the moist genital area, where MCV infections/symptoms may be atypical and difficult to differentiate from those of HSV (8, 11). Consequently, virus laboratories may encounter specimens for viral culture that contain MCV.

Although the general perception is that MCV cannot be cultured (3, 13, 17, 20), the virus was shown to produce CPE in primary cell culture in the early to mid-1960s (5, 7, 15). The CPE produced by MCV in these primary cells can be confused with that produced by HSV (8, 11). The virus will infect human primary fibroblast cells lines, including MRC-5 and HEPM (4, 14), HaCaT keratinocytes (14), and some other primary cells (5, 7, 15); however, infection is abortive and most studies have reported that the organism cannot be serially passaged to uninfected cells (4, 8, 11, 14, 15). A single study reported the serial passage of the MCV in amnion cells for a few cycles (7), but the results were not duplicated.

In the present case, a CPE in human fibroblast lines but not in other cells, an effect that was similar the CPE produced by HSV and could not be serially propagated, was suggestive of MCV. After excluding more common etiologies, we chose to confirm our suspicion of MCV by electron microscopy. This report illustrates the value of using multiple cell lines for the recovery of viruses from herpetic or atypical lesions. The number and type of cell line(s) utilized for HSV cultivation may be influenced by economic considerations or the belief that the use of a single cell line for the HSV detection is sufficient. Clinical laboratories are under considerable pressure to minimize costs (10, 19), and some laboratories have reduced or eliminated the use of viral culture all together. Laboratories that commonly utilize a single cell line such as A549 or Vero

cells specifically for HSV cultivation would likely fail to identify MCV, as would laboratories that rely solely on molecular methods for HSV diagnosis. Likewise, laboratories that report results based upon CPE alone may mistakenly attribute CPE caused by MCV to HSV. The potential for misdiagnosis underscores the importance of evaluating potential specimen toxicity and using additional testing methods such as FA testing to confirm that CPE suggestive of HSV is indeed due to HSV. This report also highlights the importance of the inclusion of MCV in the differential diagnosis of genital infections. An active sexual history can lead to infection with either HSV or MCV, and a negative viral culture does not necessarily rule out genital herpes, nor does it rule out MCV infection. Providers may opt for an erroneous clinical diagnosis of HSV disease, albeit not culture confirmed, and implement ineffective treatment strategies. Such a misdiagnosis might also invoke undesirable social consequences. Finally, the authors hope that this case report increases awareness among laboratory professionals and clinicians that, while MCV cannot be serially cultivated or produce infective progeny, it can be recovered in cell culture, where it may generate an abortive infection and a CPE in some cell lines that may be confused with that of HSV.

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